locomotion. Ccr2 null mice were protected from these locomotion decreases, despite similar joint damage at 8 weeks post DMM, and Ccr2 null DRG did not produce MCP-1. Therefore, we sought to further investigate the expression and regulation of MCP-1 in the DMM model. The current goals were to: 1) Test whether TNF-α induces MCP-1 production in DRG cells, since it has been shown that TNF-α induces Mcp-1 mRNA in cultured DRG cell lines. 2) Analyze MCP-1 protein content in the knees of wild-type (WT) and Ccr2 null mice after DMM.

Methods: Knee-innervating DRG, L3-L5, were collected from 10-week old naïve C57BL/6 WT or Ccr2 null mice. Cells were isolated and cultured for 2 days in basal medium before 48 h-stimulation with 0, 25, or 100 ng/mL TNF-α; supernatants were collected for MCP-1 ELISA. Hip cartilage explants were harvested from 5-week old naove C57BL/6 mice. Hip cartilage was used since it is not possible to culture mouse knee cartilage explants. Explants were rested overnight, stimulated with 100 ng/mL TNF-α for 48 h, and supernatants were collected for MCP-1 ELISA. DMM or sham surgery was performed in the right knees of 10-week old male C57BL/6 WT or Ccr2 null (Taconic #3736) mice. At 0, 4, and 8 weeks after surgery, whole knee joints were extracted for ELISA. At 4 or 8 weeks post surgery, DRG cells were cultured for 4 days and supernatants collected for ELISA.

Results: In order to test whether TNF-α is able to stimulate primary DRG cells to produce MCP-1 protein, we treated naïve WT DRG cells with TNF-α. Cultures stimulated with 25 ng/mL TNF-α produced 37-fold increased amounts of MCP-1 compared to unstimulated cells ($p<0.0001$); a higher concentration of 100 ng/mL TNF-α did not further increase MCP-1. This confirms earlier reports of increased Mcp-1 mRNA following TNF-α stimulation. DRG cells from naïve Ccr2 null mice responded in the same way to TNF-α.

We looked for the presence of TNF-α in cultures of DRG cells taken from WT or Ccr2 null naïve, sham, or DMM mice at 4 and 8 weeks post surgery. None of these cultures contained measurable amounts of TNF-α.

Next, we determined MCP-1 levels in whole knee joint extracts after DMM in WT and Ccr2 null mice. In WT, knee MCP-1 protein levels were elevated 4 weeks post DMM, compared to sham and naïve age-matched controls ($p<0.0001$); by 8 weeks post DMM, levels had returned to baseline. MCP-1 was also increased in knee extracts from Ccr2 null mice, 4 weeks after DMM, but to a lesser extent than in WT. In order to determine the potential source of MCP-1 in the knee, we began by testing whether cartilage is able to produce MCP-1. Cartilage explants stimulated with 100 ng/mL TNF-α produced 86-fold increased levels of MCP-1 compared to unstimulated explants ($p=0.0008$). When we looked for the presence of TNF-α in knee extracts, however, we found that DMM and naïve extracts contained similar low levels of TNF-α at 4 and 8 weeks post surgery. TNF-α levels in Ccr2 null knee extracts mirrored the WT results, with no difference at 4 or 8 weeks between DMM and naïve extracts.

Conclusions: These observations confirm that TNF-α can stimulate DRG neurons and cartilage explants to produce MCP-1 protein, confirming previous reports that TNF-α induces MCP-1 mRNA in these tissues. We did not, however, detect elevated TNF-α levels in the DRG or in the knee joint in situ in the DMM model at 4 or 8 weeks post surgery. It is possible that TNF-α upregulation occurs at earlier time points than the ones studied here (McNamee et al 2010 reported TNF-α mRNA in the knee at 3 days post DMM) or that other factors are driving MCP-1 production.

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FINGER LENGTH PATTERN AS A BIOMARKER FOR PRENATAL ANDROGEN EXPOSURE AND THE RISK FOR OSTEOARTHRITIS AND PAIN

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Purpose: The prevalence of type 3 fingerlength pattern, determined by a shorter 4th digit compared to the 2nd digit, is influenced by prenatal androgen exposure and has been studied previously as a biomarker for gender differences in the risk of several traits. Osteoarthritis (OA) and chronic musculoskeletal pain are both sexually dimorphic. In this study, we evaluate the association of fingerlength type, as a marker of prenatal androgen exposure, with the risk of osteoarthritis (OA) and chronic musculoskeletal pain.

Methods: Fingerlength pattern was determined in a total of 4784 participants of the prospective cohort Rotterdam Study II and III. Hand X-rays were visually classified in three different fingerlength pattern types. Logistic regression was used to analyze the association of type 3 fingerlength pattern with radiological OA of the hip, knee and hand. A meta-analysis of previous published studies and our results was performed to evaluate the association with kneeOA. Subsequently, we studied the association of type 3 fingerlength pattern with chronic musculoskeletal pain.

Results: Participants with type 3 fingerlength pattern had a 64% increased risk for having handOA (OR 1.64; P-value 1.06*10-7). This finding was independent of the severity of the disease. No associations with knee- or hipOA were found. The meta-analysis of kneeOA and type 3 fingerlength pattern showed no evidence for association, however large heterogeneity was observed, which was probably driven by differences in the OA phenotype definition. Only studies that had a clinical definition, defined by both structural damage to the knee and pain, showed an association between knee OA and fingerlength patterns. We therefore examined whether type 3 fingerlength pattern was associated with chronic joint pain and, we indeed observed that individuals with a lower 2D:4D ratio had 41% more risk for having joint pain (OR 1.41; P-value 1.4*10-3).

Conclusions: Type 3 fingerlength pattern is associated with handOA, indicating prenatal androgens to contribute to the development of this disease. We additionally observed an association between low 2D:24 ratio and the risk for joint pain. Since prenatal androgens influences both the development of fingerlength pattern and the brain, a developmental cerebral susceptibility for chronic musculoskeletal pain may underlie the association found with type 3 fingerlength pattern.

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IN VIVO IMAGING OF NF-κB ACTIVITY AND CORRELATION TO PAIN IN A MODEL OF INFLAMMATORY ARTHRITIS

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Purpose: A number of pro-inflammatory and catabolic changes are observed during the progression of osteoarthritis. However, the relationship between these changes, the molecular events that regulate these changes, and the development of painful symptoms is incompletely understood. Many of the pro-inflammatory and catabolic pathways rely on the downstream effects of NF-κB activity, a key transcription factor involved in regulating inflammation. This study utilized non-invasive in vivo luminescence imaging of NF-κB activity and simultaneous measures of pain sensitivities in a mouse model of inflammatory arthritis to test for relationships between NF-κB activity and pain.

Methods: Transgenic mice engineered to carry cDNA for luciferase downstream of NF-kB response elements were acquired for this study (n=24, BALB/C-Tg(NF κB-RE-luc), age 7-8 weeks, Taconic). Mice received a 5 uL intra-articular injection of normal saline (n=12) or 10 mg/ml monoiodoacetate (MIA, n=12). A subset of mice (n=6/group; MIA, Saline) were sacrificed on day 3 post injection with the remaining mice being sacrificed on day 28 post injection. Mice underwent in vivo imaging of luminescence to measure NF-κB activity (IVIS100, Caliper) preoperatively and at intervals from day 1 to 28; mice also underwent tactile allodynia and incapacitance meter testing at these same time-points to measure a threshold for paw withdrawal from mechanical stimuli and weight bearing, respectively. The knees were saved for histology at time of sacrifice.

Results: MIA injection induced arthritic changes in the NF-κB reporter transgenic mouse model including NF-κB activity, joint degradation, and the development of pain sensitivities. NF-κB activity was significantly increased in the knee ROI after injection on days 1 and 3 compared to preoperative values, and on day 3 when compared to saline injection ($p<0.05$, 2-way ANOVA, Tukey Post Hoc) (Fig. 1a,b). The 50% withdrawal threshold was significantly decreased for MIA injection on days 3 and 7 compared to preoperative values, and significantly decreased on days 3, 7, 21, and 28 when compared to saline injection ($p<0.05$, 2-way ANOVA, Tukey Post Hoc) (Fig. 1c). Weight distribution significantly shifted to the contralateral limb for MIA injection animals on days 3 and 7 when compared to pre-operative values ($p<0.05$) and on day 3 when compared to Saline injection ($p<0.05$) (Fig. 1d). A

relationship between NFκB activity and mechanical allodynia was observed with a Spearman ρ of -0.69 ($p < 0.0001$) (Fig. 1e). NF-κB activity was moderately correlated to weight distribution with a Spearman ρ of -0.39 ($p = 0.0002$).

Conclusions: This study demonstrates the use of non-invasive luminescence in vivo imaging to measure NF-κB activity in a mouse model of osteoarthritis and the comparison of a key molecular event in arthritis to pain sensitivity development. MIA injection induced a transient increase in NF-κB activity at early time points in the intra-articular joint that was correlated with developing pain sensitivities in this model of arthritis. The demonstrated relationship between NFκB activity, mechanical allodynia, and weight bearing suggests the use of NFκB luminescence imaging as a novel imaging biomarker of joint dysfunction in this model.

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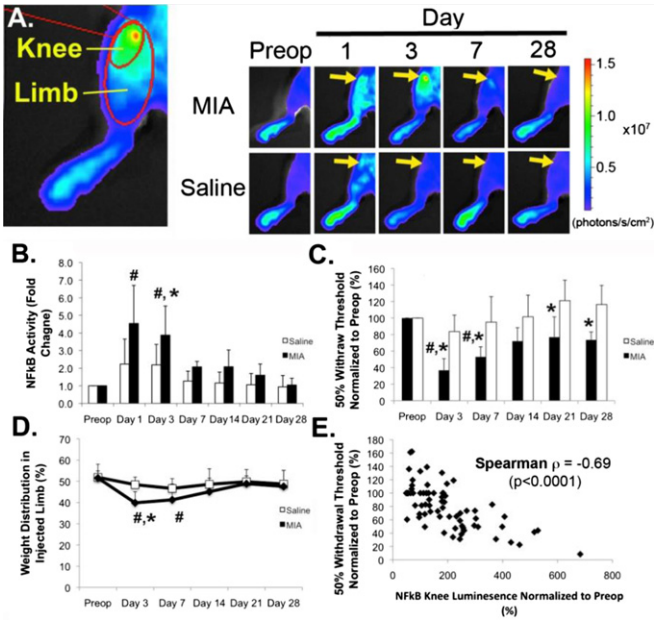

($p<0.05$ compared to preop (#) and sham (*))

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A PHASE 3 STUDY OF LOWER-DOSE DICLOFENAC SUBMICRON PARTICLE CAPSULES DEMONSTRATES EFFECTIVE PAIN RELIEF IN PATIENTS WITH OSTEOARTHRITIS

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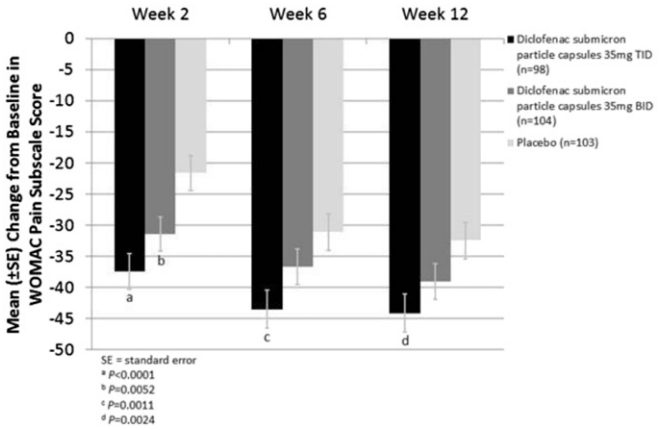
Purpose: Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for managing osteoarthritis (OA) pain; however, their use is limited by potentially serious dose-related gastrointestinal, cardiovascular, and renal adverse events (AEs). A United States Food and Drug Administration Public Health Advisory recommended that NSAIDs be administered at the lowest effective dose for the shortest duration. Investigational submicron NSAIDs are being developed using proprietary SoluMatrix™ technology that could provide effective pain relief at lower doses than currently available oral prescription NSAIDs. Diclofenac submicron particle capsules previously demonstrated analgesia in a post-surgical model of mild to moderate pain. This Phase 3 study evaluated diclofenac submicron particle capsules in patients with OA pain.

Methods: This randomized, multi-center, double-blind, parallel-group study enrolled 305 patients 41-90 years of age with OA of the hip or knee. Patients had Kellgren-Lawrence grade II-III radiographic OA severity, were chronic NSAID or acetaminophen users, with baseline WOMAC pain subscores ≥50mm, that increased by ≥15mm following NSAID discontinuation. Patients were randomized to diclofenac submicron particle capsules 35mg TID or 35mg BID, or placebo. The primary endpoint was mean change from baseline in WOMAC pain score at week 12.

Results: Most patients were women (203 [66.6%]) and Caucasian (245 [80.3%]) with a mean age of 61.6 (±8.9) years. Diclofenac submicron particle capsules 35mg TID demonstrated significant pain improvement (-44.1; $P=0.0024$; **Figure**) compared with placebo (-32.5) at week 12. There was numerical evidence of pain improvement for diclofenac submicron particle capsules 35mg BID at week 12, although this did not achieve statistical significance (-39.0; 95% confidence interval [CI], -33.3 to -44.8; $P=0.0795$). Patients receiving diclofenac submicron particle capsules 35mg TID (-37.4; 95%CI, -31.7 to -43.1; $P<0.001$) and diclofenac submicron particle capsules 35mg BID (-31.4; 95%CI, -26.0 to -36.8; $P=0.0052$) experienced improvements in WOMAC pain subscale scores at weeks 2, compared with placebo (-21.6; 95%CI, -27.1 to -16.1). At week 6, patients administered diclofenac submicron particle capsules 35mg TID (-43.5; 95%CI, -37.5 to -49.5; $P=0.0011$) maintained clinical improvements in WOMAC pain subscale scores compared with placebo (-31.1; 95%CI, -25.3 to -36.8). The average improvement in total WOMAC pain subscale scores favored diclofenac submicron particle capsules 35mg TID (-35.9; $P=0.0002$) and 35mg BID (-30.3; $P=0.0363$) compared with placebo (-23.2). A higher proportion of patients who received diclofenac submicron particle capsules 35mg TID (26.0%, 25/98) or BID (22.5%, 23/104) reported their pain as "very much improved" compared with placebo (6.3%, 6/103). The most frequent treatment-emergent AEs were similar across treatment groups and included diarrhea, headache, nausea, and upper respiratory tract infection.



Conclusions: Investigational lower-dose, diclofenac submicron particle capsules provided better overall relief from OA pain compared with placebo and were generally well-tolerated. These results suggest that diclofenac submicron particle capsules are a potentially promising therapeutic option for patients with OA pain.

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LOWER-DOSE INDOMETHACIN SUBMICRON PARTICLE CAPSULES PROVIDE EFFECTIVE ACUTE PAIN RELIEF: PHASE 3 STUDY RESULTS

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Purpose: Although often prescribed for treatment of acute pain, NSAIDs have the potential for the development of dose-related gastrointestinal, cardiovascular, and renal complications. A United States Food and Drug Administration Public Health Advisory recommended that NSAIDs should be administered at the lowest effective dose for the shortest duration consistent with individual patient treatment goals. Investigational submicron NSAIDs are being developed using proprietary SoluMatrix™ technology that could provide effective pain relief at lower doses than currently available oral NSAIDs. In a Phase 2 study, indomethacin submicron particle capsules provided good pain relief and were generally well-tolerated in a post-surgical model of mild to moderate pain. This study evaluated the analgesic efficacy and safety of lower-dose, indomethacin submicron particle capsules and celecoxib versus placebo in a validated post-surgical model of moderate to severe pain.

Methods: This multi-center, double-blind study enrolled patients 18-68 years of age who underwent a primary, unilateral first metatarsal